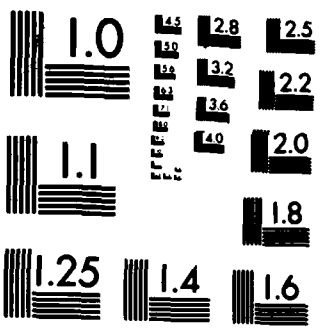
AD-A147 107 EFFECTS OF LOW TEMPERATURES ON THE GROWTH AND UNFROZEN WATER CONTENT OF AN AQUATIC PLANT(U) COLD REGIONS RESEARCH AND ENGINEERING LAB HANOVER NH 1/1

UNCLASSIFIED A J PALAZZO ET AL. JUN 84 CRREL-84-14 F/G 6/3 NL


1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8

CRREL
REPORT 84-14

(12)

US Army Corps of Engineers
Cold Regions Research & Engineering Laboratory

Effects of low temperatures on the growth and unfrozen water content of an aquatic plant

For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380, Metric Practice Guide, *published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.*

Cover: Experimental set-up for the greenhouse phase of the experiment.

CRREL Report 84-14

June 1984

Effects of low temperatures on the growth and unfrozen water content of an aquatic plant

A.J. Palazzo, A.R. Tice, J.L. Oliphant and J.M. Graham

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: CRREL Report 84-14
2. GOVT ACCESSION NO.: AD-A147 107
3. RECIPIENT'S CATALOG NUMBER:

4. TITLE *(and Subtitle)*: EFFECTS OF LOW TEMPERATURES ON THE GROWTH AND UNFROZEN WATER CONTENT OF AN AQUATIC PLANT
5. TYPE OF REPORT & PERIOD COVERED:
6. PERFORMING ORG. REPORT NUMBER:

7. AUTHOR(s): A.J. Palazzo, A.R. Tice, J.L. Oliphant and J.M. Graham
8. CONTRACT OR GRANT NUMBER(s):

9. PERFORMING ORGANIZATION NAME AND ADDRESS: U.S. Army Cold Regions Research and Engineering Laboratory, Hanover, New Hampshire 03755
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS:

11. CONTROLLING OFFICE NAME AND ADDRESS:
12. REPORT DATE: June 1984
13. NUMBER OF PAGES: 15

14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*:
15. SECURITY CLASS. *(of this report)*: Unclassified
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE:

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution is unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Aquatic plants
Aquatic weeds
Low temperature
Nuclear magnetic resonance

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Two laboratory studies were performed to investigate the effects of low temperatures on the aquatic plant *Ceratophyllum dimersum* L. Whole plants were subjected to low-temperature treatments of +4°, 0° and −6°C for 48 hours, and regrowth was compared to an untreated control. The control and +4°C-treated plants gained weight, while visible injury and reductions in plant biomass were noted 30 days after treatment at the two lower temperatures. The −6°C treatment killed the plants, while the 0°C treatment injured them to some degree. In another phase of this study, nuclear magnetic resonance (NMR) analysis of plant buds, leaves and stems showed that lowering temperatures caused the plants' unfrozen water content to drop rapidly as the temperature approached −5°C, then slowly as temperatures approached −13°C. From −13°C to −22°C there was little change in unfrozen water content. The results show that

DD FORM 1 JAN 73 1473 / EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

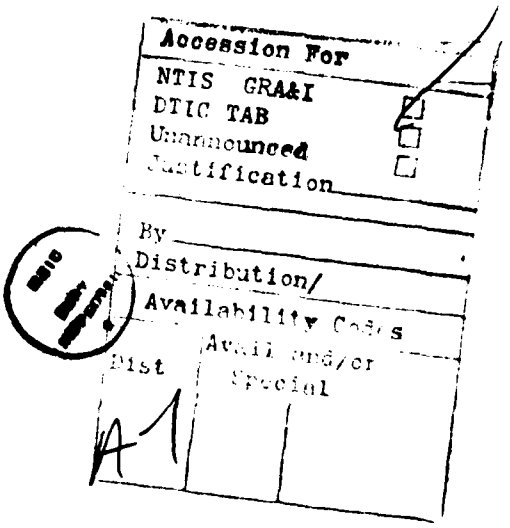
SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

20. Abstract (cont'd)

ice in this plant causes injury that affects subsequent regrowth; temperatures of -6°C or below can actually kill them. This killing temperature was also near the point where frozen water content increased only slightly with lower temperatures. NMR analysis could be one way of determining plant tolerance to cold. It appears from this study that this weedy species is susceptible to low-temperature injury, and subjecting this plant to cold may be a promising method of weed control in northern lakes.

PREFACE

This report was prepared by Antonio J. Palazzo, Research Agronomist; Allen R. Tice, Physical Science Technician; Joseph L. Oliphant, Research Physical Scientist; and John M. Graham, Biological Technician, Earth Sciences Branch, Research Division, U.S. Army Cold Regions Research and Engineering Laboratory. This research was funded under the CRREL ILIR program. The authors thank J. Filbin, Columbia College, and John Baust, University of Houston, for reviewing this report.



EFFECTS OF LOW TEMPERATURES ON THE GROWTH AND UNFROZEN WATER CONTENT OF AN AQUATIC PLANT

A.J. Palazzo, A.R. Tice,
J.L. Oliphant and J.M. Graham

INTRODUCTION

Aquatic weeds are a major nuisance in managing U.S. Army facilities. These weeds have been known to grow in sewage lagoons and potable water supplies and reservoirs. Controlling these pests with herbicides can be expensive or environmentally damaging. Where chemicals are not permitted, labor-intensive physical controls must be used.

In cold regions, water levels can be manipulated to control weedy plants in aquatic environments. In one technique, water levels were lowered in the winter to expose plants and bottom sediments to cold, which froze susceptible plants and resulted in death or reduced growth the following season (Kroll 1980). Techniques like this, if successful, could be cost-effective and environmentally safe alternatives for weed control in cold climates.

There are two primary methods for evaluating a plant's winter hardiness (its tolerance of low temperatures): its survival under field conditions and its physiological responses to cold, which are determined in the laboratory and correlated with field survival.

Previous freezing studies have shown that the degree of cold injury for aquatic plants can differ according to species, time of year, weather and conditions during acclimation to cold (Beard 1973, Dunst et al. 1974, Cooke 1980, Kroll 1980). Plants may be killed at temperatures just slightly below freezing in summer, but many survive very low temperatures in winter (Weiser 1970, MacDowall and Buchanan 1974, Harrison et al. 1978). Hardy stems of red-osier dogwood (*Cornus stolonifera* Michx.) have been reported to have survived direct immersion in liquid nitrogen (-196 °C) (Harrison et al. 1978). In nature, plants become acclimated to cold during the fall when temperatures drop slowly. The stimuli to activate acclimation processes can vary and can include temperature and light (Weiser 1970).

Cold injury to plants can occur during either slow or rapid freezing. Slow freezing causes extracellular ice formation, dehydrating the protoplasts as the water moves from the protoplasts to the extracellular ice. Injury can then be related to dehydration (Siminovitch and Briggs 1953, Levitt 1959, 1972, Chen and Gusta 1978a, Chen et al. 1978, Stout et al. 1978). Plants can resist freezing injury by either tolerating or avoiding cellular dehydration (Levitt 1959). Besides cellular dehydration, extracellular ice formation can cause mechanical damage in plants (Chen et al. 1976).

One of the first signs of damage due to rapid freezing, or frost injury, is the loss of membrane semipermeability (Levitt 1972). Rajashekar et al. (1979) attempted to determine when damage occurred during frost injury. They found that the primary site of damage is the plasma membrane

and that the damage occurs at a critical temperature during freezing. Frost injury from a brief exposure to a critical temperature is irreversible and lethal.

Rapid freezing, which occurs during nuclear magnetic resonance (NMR) analysis of water in plants, can result in intracellular ice formation (Chen and Gusta 1978a, Chen et al. 1978, Stout et al. 1978). Chen and Gusta (1978a) and others have reported that the survival rate of plants during rapid cooling depends more on the rate at which water leaves the cells or the amount of ice or water present than on the rate of cooling. Chen et al. (1976) used pulsed NMR analysis to study the freezing process in excised leaf tissue of several *Solanum* species. The major difference between the tender and hardy tissues was the ability of hardy tissue to tolerate more frozen water at each plant's killing temperature. The less-hardy tissue contained about 41–47% liquid water, while the hardier species contained 22–36% liquid water at the killing temperature. Tolerance of frozen water or diminished quantities of liquid water at low temperatures has been shown for hardy species such as cereals (Gusta et al. 1975, Chen and Gusta 1978b), dogwood (Burke et al. 1974), and *Hedera helix* (Stout et al. 1978). No correlations could be found between the amounts of liquid water and killing temperatures for several species of plants (Gusta et al. 1975, Chen and Gusta 1978b).

Rajashekar et al. (1979) reported that the use of NMR analysis alone could not detect the killing temperature of plants. This shows the importance of combining laboratory, greenhouse and field studies to evaluate plant hardiness.

The objectives of this study were to determine the amounts of unfrozen water under different temperatures in the aquatic plant *Ceratophyllum dimersum* L. and to relate these temperatures to plant injury or death. The amounts of unfrozen water and ice in various plant tissues were determined using nuclear magnetic resonance (NMR). This technique has been successful for obtaining curves for phase composition vs temperature in frozen soils, and we tested its usefulness for obtaining these curves in living cells (Tice et al. 1982). Whole plants were subjected to low temperatures in environmental chambers, and subsequent growth was measured for injury.

MATERIALS AND METHODS

Samples of the aquatic plant *Ceratophyllum dimersum* L. were shipped cold to CRREL from Eau Galle Reservoir in Wisconsin in February 1982. This plant is the dominant submerged macrophyte in this lake and in places grows to noxious levels.* The plants were cultured in the CRREL greenhouse in plastic pans containing 8 L of water taken from Post Pond in Lyme, N.H.

The water had an alkalinity of 17 mg/L and a pH of 6.0. To increase these to the approximate levels of the plants' natural environment, we added one gram each of $CaCO_3$ and K_2CO_3 to each basin. These additions increased the alkalinity to 98.2 mg/L and the pH to 8.9. To evaluate the suitability of the pond water as an adequate environment for the plants, we tagged five plant samples and periodically recorded their growth for 30 days. Gains in plant fresh weight were recorded during this period.

The plants received supplemental lighting with fluorescent lights 12 cm above the basin. The daytime temperature of the water in the basins and the maximum and minimum temperatures of the greenhouse were periodically measured. The average daily temperature from all measurements was 20.4 °C, with a range of 14.8–28.3 °C. During sunny afternoons the basins were shaded with cheesecloth.

For NMR analysis whole plants were removed from the basins and carefully blotted to remove excess water. Bud, leaf and stem tissue was separated and placed in individual glass test tubes 19 mm O.D. × 150 mm high. A copper-constantan thermocouple was placed in the center of each sample to measure temperature. The test tubes were then sealed with rubber stoppers to prevent moisture changes. After the samples were prepared, the test tubes were placed in a precision temperature bath containing a mixture of ethylene glycol and water, and they were allowed to equilibrate at the first test temperature of 18.4 °C.

The pulsed NMR used in this investigation was a Praxis model PR-103 operated in the 90° mode with a 0.2-s clock and a fast scan speed. First-pulse amplitudes (signal intensities) were measured for each sample. The operating frequency is 10.72 MHz. The sample probe contains a 2.51-kilogauss permanent magnet. The 90° pulse length is 12 μs followed by a dead time of 24 μs. We selected this mode of operation over other available modes because of the nonadjustable dead time of 24 μs. Signals from hydrogen associated with ice and that associated with solid ($T_2 < 24$ μs) would be lost in the recovery time between the 90° pulses.

*Personal communication with J. Filbin, Columbia College, 1982.

We performed tests to ensure that we were measuring hydrogen associated with liquid water only. Large single crystals of pure ice were machined to fit into glass test tubes. Single crystals of ice were selected instead of polycrystalline ice to guard against any signal contributions from liquid films between ice grain boundaries. The crystals of ice were placed in a bath maintained at -0.3 °C. No signal other than the background level was observed when the crystals of ice were inserted in the NMR. Signals are rarely observed from oven-dry (105 °C) solids. If a signal was observed in the oven-dry solids, we subtracted this value from all readings prior to calculating unfrozen water contents.

At the first test temperature following thermal equilibration, a background reading on the NMR spectrometer was recorded, and the test tubes were sequentially removed from the bath, wiped dry, and measured in the NMR analyzer. The sample temperature and NMR signal amplitude were recorded, and the samples were reinserted in the bath; the elapsed time was about 4 s.

After all the samples had been analyzed, the bath temperature was lowered by approximately 3 °C. The samples were allowed to equilibrate at the new temperature for a minimum of 30 minutes, and the measurements were repeated. NMR signal intensities were recorded at nine above-0 °C temperatures. These readings, when plotted vs temperature, constitute the paramagnetic regression line (Tice et al. 1982) and are the baseline for calculating unfrozen water contents.

Following the above-0 °C readings, the samples were cooled to about -0.7 °C and frozen by nucleation with a needle dipped in liquid nitrogen. After equilibration at this temperature, a reduction in signal intensity, corresponding to the amount of water present as ice, was recorded for each sample. The bath temperature was then reduced and the procedure repeated until a complete cooling curve was attained. Following the determination at about -21 °C, the procedure was reversed, and a complete warming curve was attained, including those above-0°C measurements that form the paramagnetic regression line. Following the last warming determination the stoppers were removed and the water contents were determined gravimetrically after drying at 105 °C.

The raw NMR data from the cooling and warming runs are shown in Fig. 1. The first-pulse amplitude increases as the temperature decreases for the samples containing no ice, but the first-pulse amplitude is reduced when water begins to freeze just below 0 °C. Unfrozen water contents are calculated by drawing a line through the data points above 0 °C and extending it to low temperatures by linear regression. The unfrozen water content is then calculated as the total water content (determined gravimetrically) multiplied by the distance from the experimental measurement to the background amplitude and then divided by the distance from the regression line to the background amplitude reading (Tice et al. 1982). Figure 1 shows that the paramagnetic lines for warming are much higher than those for cooling. Therefore, the unfrozen water content for the cooling runs was calculated from the regression for the cooling data, and the content for the warming runs was calculated from the warming data.

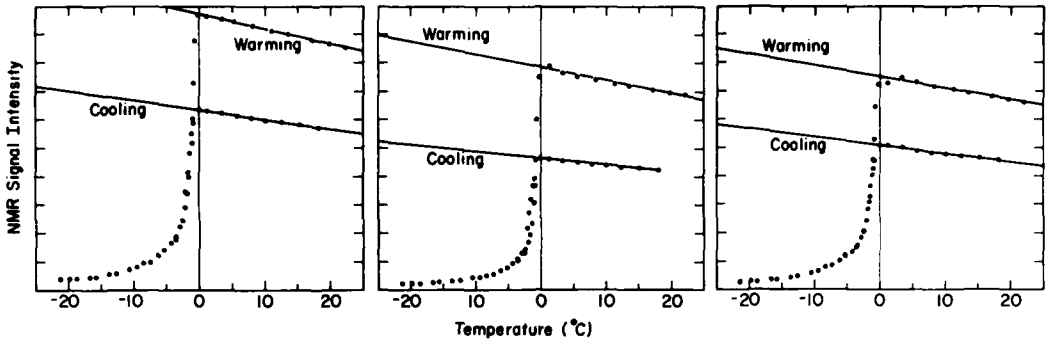
After the NMR analyses were completed in June, we began the second phase of this study, which was to subject *Ceratophyllum* plants to low temperatures. Before we could begin, most of the plants died. These plants were replaced with others obtained from the Eau Galle Reservoir.

The reason for the death of these plants is not clear, but it could be partially related to the high temperatures in the basins and the greenhouse (Table 1). Basin temperatures increased approximately 7 °C during the day, and the temperature was higher than 24 °C for 6 of the 10 readings. Carr (1969) observed rapid declines in the rate of photosynthesis in *Ceratophyllum* as temperatures increased above 20 °C. She also found that the rate of photosynthesis at 30 °C was 50% of that at the optimum temperature of 20 °C.

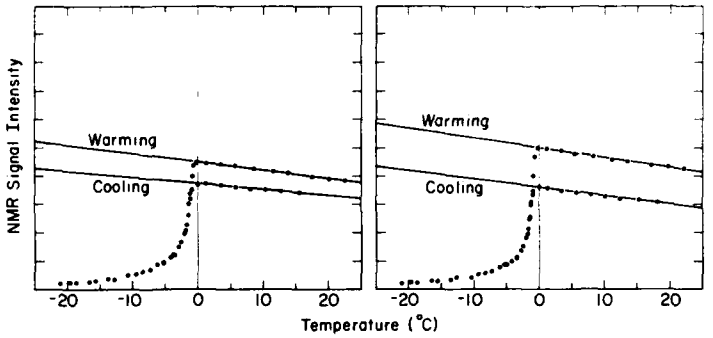
The plants were cut into 3-cm-long segments. Each segment contained at least one bud. Eight segments were tested for each replication, and there were four replications for each treatment. The fresh weights of plants in each replication

Table 1. Basin and greenhouse temperatures measured periodically between 11 March and 20 May 1982.

	Daytime basin temp (°C)		*Greenhouse temp (°C)*	
Date	*Mean*	*Range*	*Max*	*Min*
11 March	25.6	20.7-28.3	38	18
17 March	18.6	16.5-21.5	30	18
25 March	19.6	18.3-21.5	38	20
30 March	19.5	15.8-25.8	31	19
8 April	19.9	15.6-24.9	34	19
14 April	22.7	20.3-25.2	29	19
29 April	20.6	15.2-25.6	33	19
6 May	17.8	14.8-22.3	—	—
13 May	21.1	18.1-24.5	—	—
20 May	18.4	15.3-22.1	—	—



a. Stems; 801.66% water. *b. Stems; 716.63% water.* *c. Buds; 1158.16% water.*



d. Leaves; 990.14% water. *e. Leaves; 930.34% water.*

Figure 1. NMR signal intensity vs temperature for Ceratophyllum.

were measured. Samples were prepared for weighing by gently blotting excess water off of the plant surface. The test plants were subjected to temperatures of 4°, 0°, -6°C or the ambient temperature for 48 hours. After they were treated, the plants were allowed to grow. After 30 days the plants were harvested, weighed for fresh weight, and dried to constant weight to obtain oven-dry weights.

A statistical analysis was performed on the plant growth data by using an analysis of variance followed by a Duncan's Multiple Range test at the 5% level of significance.

RESULTS AND DISCUSSION

NMR analysis

The NMR readings reveal large differences above 0°C (Fig. 1), which shows the paramagnetic effect (effect of temperature on the NMR signal) for cooling and warming runs. (In soil-water systems we do not see any difference between these readings, even after several freeze–thaw cycles.) One hypothesis that might explain these differences is that two different orders of water exist in biological systems (Klotz 1958, Cope 1969, Hazelwood et al. 1969, Burke et al. 1974, Gusta et al. 1975). These authors used NMR to study water in animal tissue and reported that cell water exists in at least two observable degrees of order.

When the plants were frozen and then thawed, there was a pronounced increase in the above-0°C NMR readings (Fig. 1). This phenomenon has been observed by Rajashekar et al. (1979), who observed two separate relaxation events for live wheat leaf segments. Following freezing and rewarming they found only one major relaxing component. The large differences between the cooling and warming data may be a result of alterations in the permeability of the plasma membrane. These alterations may occur when substantial amounts of water migrate to growing ice crystals in the extracellular spaces during cooling. The larger dif-

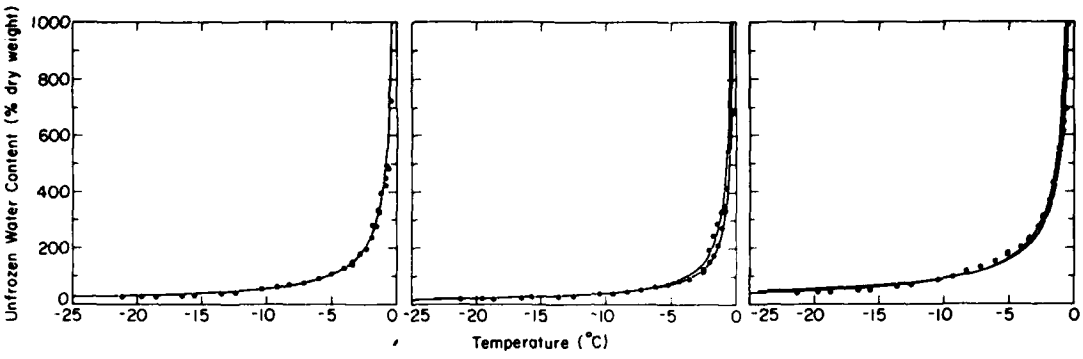
Table 2. Unfrozen water content vs temperature for *Ceratophyllum*.

Temperature (°C)	*Unfrozen water (% dry wt)*	*Temperature (°C)*	*Unfrozen water (% dry wt)*
Stems; cooling; 802% water*		Stems; warming; 802% water	
- 0.72	739.8	-21.26	25.07
- 1.03	691.8	-18.69	26.88
- 1.34	602.1	-15.73	32.59
- 1.53	515.6	-12.61	41.57
- 1.95	429.9	- 9.38	61.82
- 2.42	299.3	- 7.28	74.37
- 2.89	276.0	- 5.2	109.5
- 3.49	231.4	- 3.52	139.6
- 4.22	201.8	- 2.44	195.5
- 5.14	172.4	- 2.0	232.6
- 6.12	145.6	- 1.66	271.3
- 8.42	111.0	- 1.42	320.6
-10.58	84.4	- 0.95	419.6
-13.66	61.7	- 0.88	489.3
-16.65	49.1	- 0.77	595.3
-19.78	42.6	- 0.56	721.4
-21.26	38.9		
Stems; cooling; 717% water		Stems; warming; 717% water	
- 0.8	603.4	-21.4	15.5
- 1.11	565.1	-18.77	15.7
- 1.42	489.6	-15.79	21.9
- 1.68	414.5	-12.58	26.5
- 2.05	330.0	- 9.51	35.7
- 2.44	194.8	- 7.34	46.7
- 2.83	192.9	- 5.25	67.7
- 3.57	163.0	- 3.54	90.0
- 4.33	137.9	- 2.47	124.0
- 5.17	112.9	- 2.0	148.9
- 6.2	98.7	- 1.66	171.9
- 8.42	72.1	- 1.37	208.6
-10.55	59.5	- 1.01	275.7
-13.74	41.0	- 0.85	330.8
-16.6	33.2	- 0.69	414.4
-19.78	28.5	- 0.56	545.6
-21.4	26.8	- 0.15	685.5
Buds; cooling; 1160% water		Buds; warming; 1160% water	
- 0.67	1029.3	-21.31	33.3
- 0.93	959.4	-18.75	36.6
- 1.29	818.8	-15.71	45.7
- 1.5	690.6	-12.58	65.2
- 2.0	545.9	- 9.41	91.3
- 2.44	445.2	- 7.26	125.0
- 2.89	405.7	- 5.22	171.4
- 3.46	341.5	- 3.57	224.4
- 4.2	293.1	- 2.44	311.2
- 5.17	260.4	- 1.97	363.9
- 6.15	217.0	- 1.68	428.8
- 8.34	166.3	- 1.37	495.4
-10.47	118.9	- 0.98	614.8
-13.63	82.9	- 0.85	699.8
-16.63	67.0	- 0.72	812.6
-19.7	53.6	- 0.56	992.0
-21.31	49.0	- 0.15	1119.1
Leaves; cooling; 990% water		Leaves; warming; 990% water	
- 0.69	926.8	-21.09	33.1
- 1.06	887.5	-18.64	39.5
- 1.19	793.6	-15.6	54.3
- 1.4	686.3	-12.67	67.5
- 1.76	544.2	- 9.33	108.6
- 2.55	430.7	- 7.2	143.9
- 2.83	382.6	- 5.12	195.1
- 3.7	317.7	- 3.52	253.4
- 4.15	285.4	- 2.44	353.3
- 5.25	229.0	- 1.97	420.0
- 6.09	217.6	- 1.66	491.0
- 8.29	158.6	- 1.37	564.4
-10.53	118.8	- 0.98	691.3
-13.6	84.5	- 0.85	766.8
-16.55	56.3	- 0.72	871.1
-19.59	48.2	- 0.56	955.9
-21.09	40.6		
Leaves; cooling; 930% water		Leaves; warming; 840% water	
- 0.77	895.5	-21.26	27.9
- 0.98	857.5	-18.8	31.6
- 1.19	762.4	-15.71	42.4
- 1.4	652.0	-12.77	63.9
- 1.79	497.1	- 9.43	84.7
- 2.47	376.0	- 7.42	103.7
- 2.86	338.9	- 5.41	152.2
- 3.46	281.0	- 3.52	194.5
- 4.36	241.0	- 2.55	277.1
- 5.12	206.8	- 2.05	333.6
- 6.2	185.0	- 1.68	390.2
- 8.42	127.8	- 1.5	455.8
-10.5	91.9	- 1.11	567.0
-13.66	68.5	- 0.95	642.4
-16.63	55.5	- 0.85	741.8
-19.78	42.9	- 0.61	868.4
-21.26	38.0		

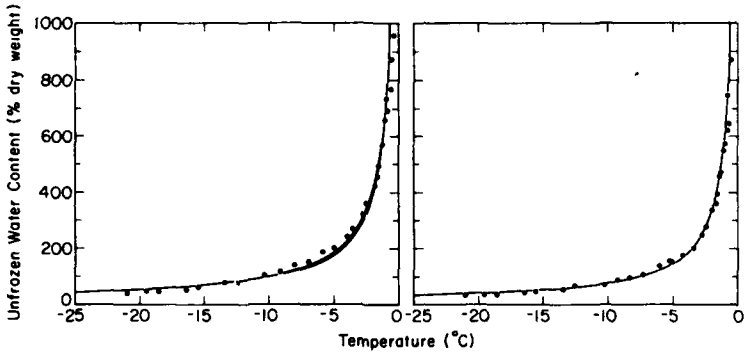
*Weight of plant water/weight of plant material.

ferences shown in Figure 1 amount to 35, 41, 33, 17 and 28% and translate into 2.80, 2.94, 3.82, 1.68 and 2.60 g of H_2O per g of dry plant material, respectively. It has also been suggested that one of the phases of water complexes with the proteins, creating hydration shells with properties that are more ice-like than liquid-like (Klotz 1958, Cope 1969).

The unfrozen water content vs temperature data are shown in Table 2 and Figure 2. At a temperature of about -21 °C the unfrozen water content ranges from 0.26–0.49 g of H_2O per g of dry plant



a. Stems; 801.66% water. *b. Stems; 716.63% water.* *c. Buds; 1158.16% water.*



d. Leaves; 990.14% water. *e. Leaves; 930.34% water.*

Figure 2. Unfrozen water content vs temperature for Ceratophyllum.

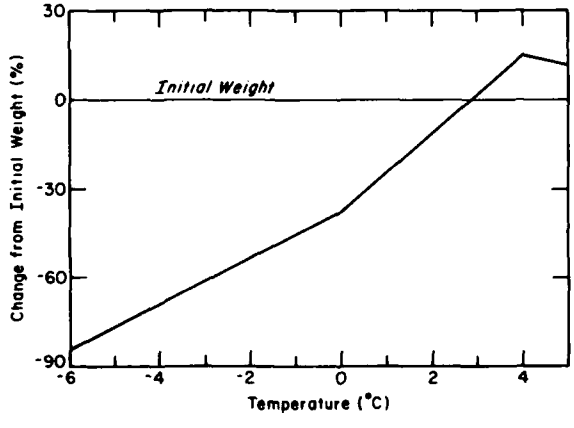


Figure 3. Changes in fresh weight of Ceratophyllum *after being subjected to low temperatures.*

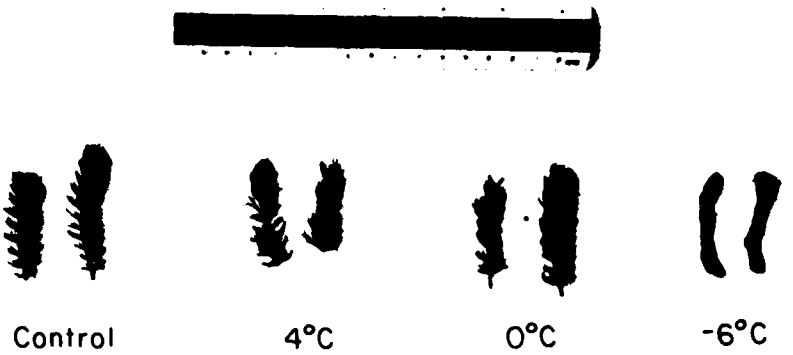


Figure 4. Appearance of selected plants 30 days after treatment.

material. This variation is in agreement with results reported by Gusta et al. (1975) and Burke et al. (1974), who reported values ranging from 0.2 to 0.4 g of H_2O per g of dry tissue. They attributed this amount to bound water that is closely associated with macromolecules and not available for freezing.

Figure 2 shows a large reduction in plant unfrozen water content as the temperatures were lowered from 0° to -6 °C, which indicates that at -6 °C most of the water is frozen. Since there was only a small increase in frozen water content below -13 °C, test temperatures selected for the next phase of the study were 4°, 0° and -6 °C along with a control.

Greenhouse study

Figure 3 shows the regrowth of plants as a percentage of their initial weight 30 days after being subjected to low temperatures. The plants subjected to temperatures of 0° and -6 °C lost weight during this time and weighed only 62% and 16%, respectively, of their initial weights.

Various degrees of cold injury were evident at the temperatures selected (Fig. 4). The control and 4 °C plants appeared normal, but the plants treated at 0 °C were brownish at the leaf tips and the -6 °C plants treated were mostly dead. The severity of the injury to the 0 °C plants could not be determined from this study. A longer regrowth period would have provided a better measure of this injury. Filbin* also found that *Ceratophyllum* was killed when subjected to a temperature of -10 °C.

*Personal communication, Columbia College.

SUMMARY

The aquatic plant *Ceratophyllum dimersum* L. has been tested for susceptibility to cold injury by measuring the unfrozen water content of plant parts using NMR and by subjecting whole plants to temperatures of +4°, 0° and -6 °C for 48 hours. During the 30-day regrowth period the plants subjected to +4 °C continued to grow the same as the controls. The plants subjected to 0 °C had visible damage and a loss of weight; from this study it cannot be determined whether these plants would recover from this treatment. Plants subjected to -6 °C were killed.

It was also found in the NMR analysis that after the plants had been frozen and subsequently thawed, there was more water that appeared to be in a liquid state than before they were frozen. The freezing process may have freed water molecules that had previously been associated with organic molecules in the plants and thus held in an ice-like state. The strength of this association could be determined from the freezing temperature at which the water is freed.

LITERATURE CITED

Beard, T.D. (1973) Overwinter drawdown: Impact on the aquatic vegetation in Murphy Flowage, Wisconsin. Wisconsin Department of Natural Resources, Technical Bulletin No. 61.

Burke, M.J., R.G. Bryant and C.J. Weiser (1974) Nuclear magnetic resonance of water in cold acclimating red osier dogwood stem. *Plant Physiology*, **54:** 392-398.

Carr, J.L. (1969) The primary productivity and physiology of *Ceratophyllum dimersum*. II. Micro primary productivity, pH, and the P/R ratio. *Australian Journal of Marine Freshwater Research*, **20**: 115–126.

Chen, P.M., M.J. Burke and P.H. Li (1976) The frost hardiness of several *Solanum* species in relation to freezing of water, melting point depression, and tissue water content. *Botanical Gazette*, **137**: 313–317.

Chen, P.M., P.H. Li and M.J. Burke (1977) Induction of frost hardiness in stem cortical tissues of *Cornus stolonifera* Michx. by water stress. I. Unfrozen water in cortical tissues and water status in plants and soils. *Plant Physiology*, **59**: 236–239.

Chen, P.M. and L.V Gusta (1978a) Changes in membrane permeability of winter wheat cells following freeze-thaw injury as determined by nuclear magnetic resonance. *Plant Physiology*, **61**: 878–882.

Chen, P.M. and L.V. Gusta (1978b) The role of water in cold hardiness of winter cereals. In *Plant Cold Hardiness and Freezing Stress* (P.H. Li and A. Sakai, Ed.). New York: Academic Press. pp. 165–197.

Chen, P.M., L.V. Gusta and D.G. Stout (1978) Changes in membrane permeability of winter wheat cells following freeze–thaw injury as determined by nuclear magnetic resonance. *Plant Physiology*, **61**: 878–882.

Cooke, G.D. (1980) Lake level as a macrophyte control technique. *Water Resources Bulletin*, **16**: 317.

Cope, F.W. (1969) Nuclear magnetic resonance evidence using D_2O for structure water in muscle and brain. *Biophysical Journal*, **9**: 303–319.

Dunst, R.C., S.M. Born, P.D. Uttormark, S.A. Smith, S.A. Nichols, J.O. Peterson, D.R. Knaver, S.L. Serns, D.R. Winter and T.L. Wirth (1974) Survey of lake rehabilitation techniques and experience. Wisconsin Department of Natural Resources, Technical Bulletin No. 75.

Gusta, L.V., M.J. Burke and A.C. Kapoor (1975) Determination of unfrozen water in winter cereals at subfreezing temperatures. *Plant Physiology*, **56**: 707–709.

Harrison, L.C., C.J. Weiser and M.J. Burke (1978) Freezing of water in red-osier dogwood stems in relation to cold hardiness. *Plant Physiology*, **62**: 899–901.

Hazelwood, C.F., B.L. Nichols and N.F. Chamberlain (1969) Evidence for the existence of a minimum of two phases of ordered water in skeletal muscle. *Nature*, **22**: 747–750.

Klotz, I.M. (1958) Protein hydration and behavior. *Science*, **128**: 135-822.

Kroll, B.L. (1980) Drawdown as a tool for lake restoration: The Sanford Lake experience. *Symposium on Surface Water Impoundments* (H.G. Stefan, Ed.). Minneapolis, Minnesota: American Society of Civil Engineers.

Levitt, J. (1959) Bound water and frost hardiness. *Plant Physiology*, **33**: 674–677.

Levitt, J. (1972) *Responses of Plants to Environmental Stresses*. New York: Academic Press.

MacDowall, F.D.H. and G.W. Buchanan (1974) Estimation of water of hydration in wintering wheat leaves by proton magnetic resonance. *Canadian Journal of Biochemistry*, **52**: 652–654.

Rajashekar, C., L.V. Gusta and M.J. Burke (1979) Membrane structural transitions: Probable relation to frost damage in hardy herbaceous species. In *Low Temperature Stress in Crop Plants, The Role of Membranes* (J.M. Lyons, D. Graham and J.K. Raison, Ed.). New York: Academic Press. pp. 255–274.

Siminovitch, D. and R.D. Briggs (1953) Studies on the chemistry of living bark of the black locust in relation to frost hardiness. III. The validity of plasmolysis and dessication tests for determining the frost hardiness of bark tissue. *Plant Physiology*, **28**: 15–34.

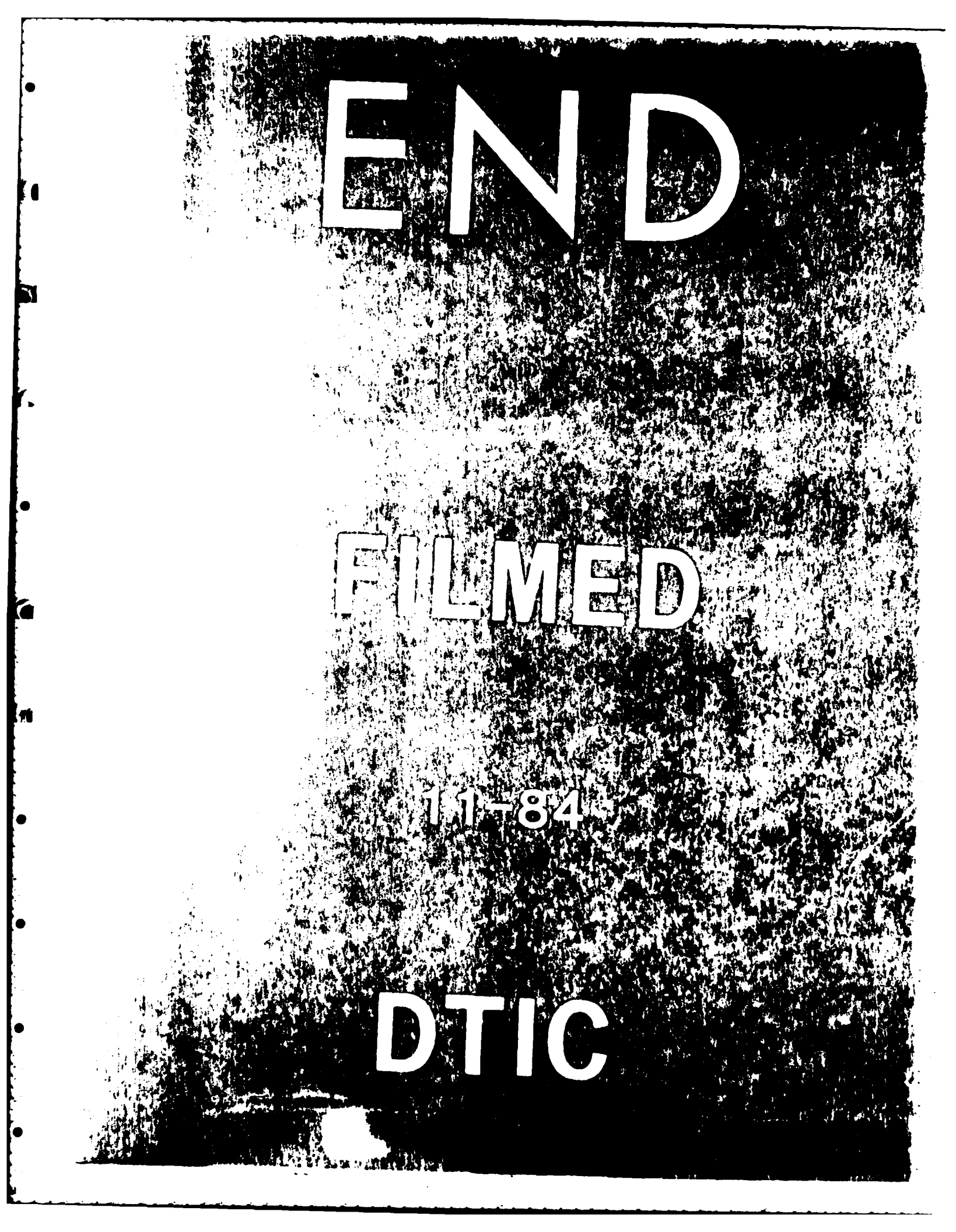
Stout, D.G., P.L. Stephonkus and R.M. Cotts (1978) Nuclear magnetic resonance relaxation times and plasmalemma water exchange in ivy bark. *Plant Physiology*, **62**: 636–641.

Tice, A.R., J.L. Oliphant, Y. Nakano and T.F. Jenkins (1982) Relationship between the ice and unfrozen water phases in frozen soil as determined by pulsed nuclear magnetic resonance and physical desorption data. USA Cold Regions Research and Engineering Laboratory, CRREL Report 82-15. ADA 118486.

Weiser, C.J. (1970) Cold resistance and injury in woody plants. *Science*, **169**: 1269–1278.

A facsimile catalog card in Library of Congress MARC format is reproduced below.

Palazzo, A.J.
Effects of low temperatures on the growth and unfrozen water content of an aquatic plant / by A.J. Palazzo, A.R. Tice, J.L. Oliphant and J.M. Graham. Hanover, N.H.: Cold Regions Research and Engineering Laboratory; Springfield, Va.: available from National Technical Information Service, 1984.
iii, 15 p., illus.; 28 cm. (CRREL Report 84-14.)
Bibliography: p. 7.
1. Aquatic plants. 2. Aquatic weeds. 3. Low temperature. 4. Nuclear magnetic resonance. I. Tice, A.R. II. Oliphant, J.L. III. Graham, J.M. IV. United States. Army. Corps of Engineers. V. Cold Regions Research and Engineering Laboratory, Hanover, N.H. VI. Series: CRREL Report 84-14.


END
FILMED
11-84
DTIC